

Evaluation of Oxidative Stress and Some Vitamins as Antioxidants in Type2 Diabetic and Nephropathic Patients.

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Abstract

Background: The present study involved the following parts, the first part is evaluation of the levels of glycated hemoglobin(HbA1c), creatinine, uric acid(UA) and albumin in patients with diabetic nephropathy comparison with the group of healthy as a control group. The second part is the measurement and evaluation of oxidative stress represented in the malondialdehyde(MDA) as a biomarker of oxidative stress as well as the identification of vitamins C and E as an antioxidant in patients with diabetic nephropathy(DN) compared with the healthy group.

Objective: The objective of this study is to estimate oxidative stress by calculate malondialdehyd as biomarker and evaluate some vitamins such as vit C and vit E as antioxidants in diabetic nephropathy patients.

Patients and Methods: This work achieved on 80 men, who attended Baghdad Teaching Hospital during first half of 2018, who were sectioned into 2 groups, 1st group involved30 healthy persons with HbA1c <6.4% as control non diabetic group, second group consisted of 50 patients with HbA1c>6.4% and creatinine >1.4 mg/dl as diabetic nephropathy patients.

Results: Results of this study showed a significant increase in DN in HbA1c, creatinine. No significant differences were shown in DN in uric acid compared to healthy control. While there was a significant decrease in DN compare to control in albumin. a significant increase in DN in MDA compare to control, while there was a significant decrease in DN compare to control in vitamins C and E.

Conclusion: The imbalance between oxidative stress and antioxidant parameters could contribute to the development of DN complications, which caused of raising the levels of HbA1c, uric acid, and creatinine.

Key words: Diabetic nephropathy, lipid peroxidation, vitamin C, vitamin E.

INTRODUCTION

A disturbance in carbohydrate, fat and proteins metabolism caused by defects in insulin secretion, insulin action, or both are an important sign of diabetes [1].

The blood sugar level can be measured and tracked in a safe and accurate manner by measuring HbA1c. Hemoglobin A1c, the most widely discovered glycosylated hemoglobin, is a glucose molecule bonded to one or the two of N-terminal valines of the β -polypeptide chains of normal adult hemoglobin [2].

Diabetic nephropathy is clinically defined by the presence of albuminuria, hypertension, and diabetic retinopathy. The development of diabetes type 2 as a result of neglect of diet and non-adherence to take medication, the patient may eventually be forced to dialysis and is a sophisticated and dangerous disease. The increase of oxidative stress leads to increased severity in patients with diabetic nephropathy. Still a research and studies on whether there is a relationship between high blood sugar and diabetic nephropathy [3].

Metabolism that occurs for creatine phosphate within muscle leads to the production of creatinine, which depends heavily on the mass of these muscles, creatinine is produced consistently. Mainly the purification of creatinine from the blood stream by the kidney, glomerular filtration and proximal tubular secretion. The level of creatinine in the blood if the kidney purification process was inefficient. To measure the creatinine clearance, it is possible to use the level of urine and creatinine in the blood, which gives an indication of the

glomerular filtration rate (GFR). Clinically, GFR is a good indicator evaluate of kidney function [4].

UA is the final product of purine metabolism. These are molecules that arise from Deoxyribonucleic acid(DNA) and ribonucleic acid (RNA) released by cells when they die. Purines obtained by catabolism of dietary nucleic acid are converted into uric acid directly. Uric acid seems to be a major protective antioxidant against NO_2 and HOCl. It has the potential for chelating iron and copper rendering them unreactive and thus inhibiting lipid peroxidation. It also reacts with singled oxygen [5].

The albumin(Alb) is made from a number of amino acids in liver, where it is produced nine to twelve grams a day without storing any excess. The albumin is high solubility in the range of 35 to 50 g per liter. It has many benefits as it transfers minerals, medicines, bile acids, the half-life of the albumin is twenty days in normal conditions. It is play important role in the regulation of osmotic pressure and distribution of fluid in the body [6].

MDA is the organic complex molecule. Its chemical formula $\text{CH}_2(\text{CHO})_2$. MDA is a biochemical indicator of lipid oxidation and as a result is an indicator of the state of the oxidative stress.

MDA exists in two chemical formulations within the body free and restricted to the thiol group and / or amino group in proteins, nucleic acids, and lipoproteins. Free MDA is the most common and chemically active form; it is a biochemical indicator of damage in the body caused by increase oxidative stress. The remaining part, which

is excreted with the urine, is the biochemical index of the old infection [7].

Vitamin C, also known as ascorbic acid and L-ascorbic acid, it is a natural product in many fruits such as lemon and orange. It can also be manufactured and used as a dietary supplement. Bio-synthesis of vitamin C in the liver is dependent on the essential substance, sugar glucose. This synthesis occurs in mammals such as guinea pigs. It acts as chain breaking antioxidant [8].

Vitamin E is a vigorous peroxy radical scavenger. α -Tocopherol is the best type of vitamin E in people, is a part of the antioxidant defense system and is a peroxy radical scavenger which keeps polyunsaturated fats inside phospholipids and plasma lipoproteins. Only the α -tocopherol from the four tocopherols and four tocotrienols found in sustenance, so human vitamin E supplement requires. Vitamin E is a vigorous peroxy radical scavenger [9].

MATERIALS AND METHODS

In the present study, 80 men, who attended Baghdad Teaching Hospital, classified into a couple of groups. First group includes 30 apparently healthy subjects {by clinical examination and with no history of diabetes (A1C <6.4%)}. Their age ranged (45 \pm 10) years. Second group included 50 patients with type 2 diabetes (A1C >6.4%), their age ranged (52 \pm 12) years diagnosed by specialized physician. According to World Health Organization (WHO), the American Diabetes Association (ADA) has suggested 5.7 – 6.4% as the high risk range, therefore we can use HbA1c levels in diagnosis of type2diabetes mellitus [10].

The blood was taken from the study groups by intravenous hole and placed in test tubes free from ethylene diamine tetra acetic acid (EDTA). The serum was separated at 3500 rpm by centrifuge. The serum is kept frozen until the time of the analysis.

Nycocard HbA1c is a boronate affinity assay traceable to the international federation of clinical chemistry and laboratory medicine (IFCC) reference method for measurement of HbA1c. The reagent contains agents called lyse erythrocytes and precipitates hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diols of glycated hemoglobin [11].

Creatinine was estimated based on its interaction with yellow- colored picric acid in alkaline medium where creatinine – picrate complex was composed of red – orange colored. Absorption was measured for the creatinine – picrate complex, which corresponds to creatinine concentration in sample.

[12].

Uric acid was measured by enzymatic colorimetric assay, using kit supplied by Agappe, Switzerland. Uric acid is oxidized by Uricase to allantoin and hydrogen peroxide [13].

The measurement of serum albumin was based on its quantitative binding to the indicator 3,3, 5,5 – tetra bromo-m- cresol, sulphonaphthalein (bromo cresol

green, BCG). The albumin –BCG-complex was absorbed maximally at λ 578 nm [14].

The concentration of MDA in serum was determined according to Buege and Aust method of enzymology. MDA formed from breakdown of poly unsaturated fatty acid (PUFA). MDA reacts with thiobarbituric acid (TBA) to give pink color that is read at λ max535nm. MDA was calculated according to the molar extinction factor of 1.56 \times 10⁵ [15].

Vitamin C and vitamin E were measured in serum, patients and healthy subjects by using high performance liquid chromatography(HPLC) [16].

Statistical Analysis

Data was compiled and analyzed using SPSSv10 software package. It was expressed as mean \pm S.D. (standard deviation), and (p < 0.05) was considered statistically significant, while (p<0.001) was considered highly significant.

RESULTS AND DISCUSSION:

Data in table (1) and Fig (1) summarize diagnostic parameters in patients and healthy subjects. Results of glycated hemoglobin showed highly significant increase (P<0.001) in sera of patients (10.74 \pm 1.75) % compared to control (5.50 \pm 0.11) %. The classification of the groups in the present study was depended on the levels of HbA_{1c} to assess a diabetic from a non-diabetic individual. It has been reported that the prevalence and overlap between intermediate hyperglycemia was defined by HbA_{1c} (5.7-6.4) %. This range was proposed as an indicator of type2 diabetes mellitus, since the advantage of using HbA_{1c} is less day to day variability compared with glucose tolerance test and fasting glucose levels [17].

serum creatinine showed highly significant increase (P<0.001) for patients (3.27 \pm 2.72) mg/dl compared to control (0.61 \pm 0.10) mg/dl.

An elevation of serum creatinine reported to be associated with a decrease in the glomerular filtration rate GFR, with progressive renal insufficiency, there is retention in the blood of creatinine which leads to increase its serum concentration [18].

Results of uric acid showed slight elevation in group of patients (5.21 \pm 2.01) mg/dl compared to control group (4.12 \pm 0.95) mg/dl, but yet the increase was not significant.

In this study, we noticed the high level of uric acid in patients with diabetic nephropathy, may inhibit stimulation of insulin by bonding with the residues of arginine acid in beta cells of the pancreas. While other studies have shown the opposite, as those studies showed that serum uric acid is inversely proportional to DN [19]. One explanation for the adverse results in studies may be associated with the inhibition of uric acid absorption due to high blood glucose levels. In general, the effect of hyperuricemia did not have more investigate in patients with DN [20].

Serum albumin showed a highly significant decrease (P<0.001) in sera of patient (3.22 \pm 0.54) g/dl compared to control (4.11 \pm 0.10) g/dl.

Table (1): Serum Levels of, HbA1c, Creatinine, Uric acid and Albumin in Sera of Studied Groups.

Studied Groups	Control	Patients	P -Values
Parameters	Mean±SD	Mean±SD	
HbA1c %	5.50±0.11	10.74±1.75	P<0.001
Creatinine (mg/dl)	0.61±0.10	3.27±2.72	P<0.001
Uric acid (mg/dl)	4.12±0.95	5.21±2.01	P>0.05
Albumin (g/dl)	4.11±0.10	3.22±0.54	P<0.001

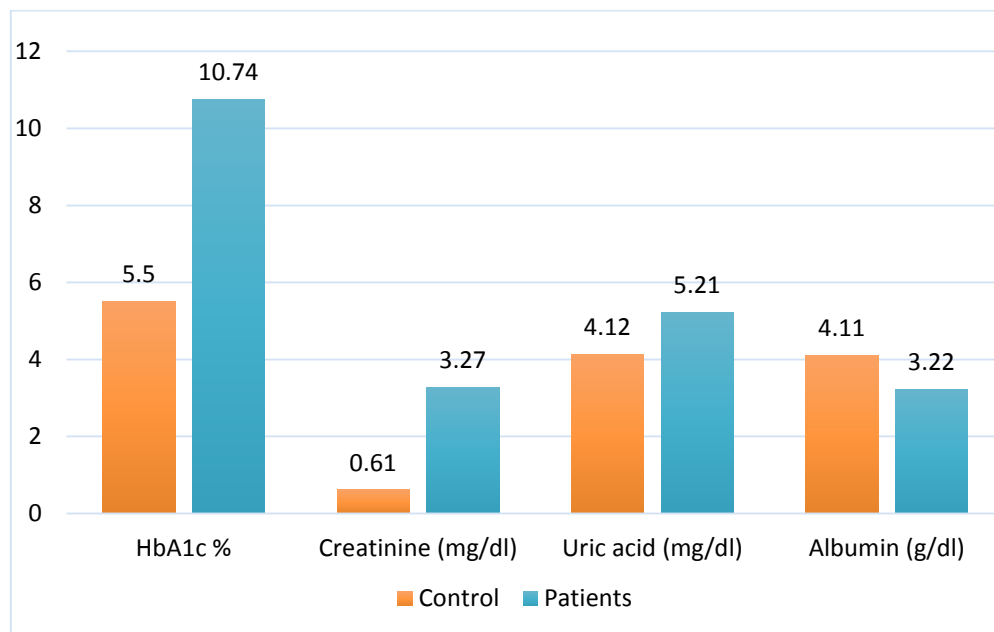


Figure (1): Serum Levels of, HbA1c, Creatinine, UA and Alb in Sera of Studied Groups.

Table (2): Serum Levels of MDA, Vitamins C and E.

Studied Groups	Control	Patients	P Values
Parameters	mean±SD	mean±SD	
MDA(μmol/L)	1.72±0.17	6.02±0.74	P<0.001
Vitamin C (mg/dl)	1.31±0.10	0.80±0.11	P<0.001
Vitamin E (mg/dl)	1.46±0.21	0.92±0.22	P<0.001

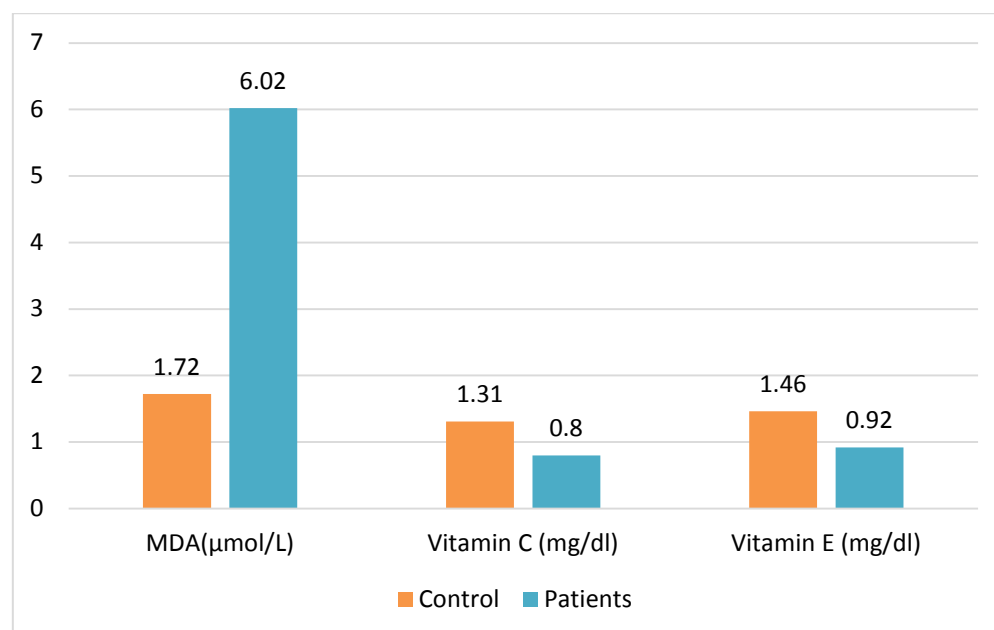


Figure (2): Serum Levels of MDA, Vitamins C and E.

Low plasma albumin concentration has significantly reduced plasma antioxidant capacity due to the diminished availability of thiol groups [21].

Albumin inhibits copper but not iron dependent hydroxyl radical generation. In addition, albumin has other antiradical effects and it might be able to scavenge some hydroxyl radicals [22].

Researchers showed impairment of the antioxidant properties of serum albumin in patients with diabetes, due to lower binding activity of glycated serum albumin to copper than that of non-glycated serum albumin [23].

Table (2) and Fig (2) shows highly significant increase ($P < 0.001$) in serum MDA level between patients (6.02 ± 0.74) $\mu\text{mol/L}$ compared to control (1.72 ± 0.17) $\mu\text{mol/L}$.

The results obtained in this work support and agree significantly with previous research and studies indicating that diabetic patients suffer from high oxidative stress due to increased free radical generation. This rise in oxidative stress leads to a disturbance between oxidant and antioxidant systems in type 2 diabetic patient's nephropathy [24].

In this study, we found that increased oxidative stress and high ROS production was due to high blood glucose level. Unlike healthy subjects. Generally, oxidative stress is more elevated in diabetic nephropathic patients than those with diabetic patients who have no complications.

High blood sugar leads to increased oxidative stress and thus increased production of mitochondrial reactive oxygen species, non enzymatic glycation of proteins and glucose autoxidation. High glucose induces intracellular ROS in mesangial and tubular epithelial cells and acting as integral glucose signaling molecules in the diabetic kidney [25].

It has been observed that increased oxidative stress increases the morbidity of diabetic nephropathy. This has been demonstrated by research on animal models both *in vitro* and *in vivo*. Mechanism of this assumption is not clear yet [26].

Table (2) and Fig (2) shows highly significant increase ($P < 0.001$) in serum vitamin C level between patients (0.80 ± 0.11) (mg/dl) compared to control (1.31 ± 0.10) (mg/dl) and serum vitamin E level between patients (0.92 ± 0.22) (mg/dl) compared to control (1.46 ± 0.21) (mg/dl) respectively.

In our study the lower levels of vitamin C and E are seen in DN patients. Our results are similar to other researchers. Vitamin C and E are diet derived and detoxify free radicals by chain breaking reaction. They also interact in recycling processes to generate reduced forms of the vitamins which may destroy free radicals. Non-enzymatic antioxidants such as vitamins C and E interrupt free radical chain reactions. Diabetic patients with nephropathy who have low levels of vitamins C and E may increase these vitamins in the serum after taking dietary supplements rich in these vitamins. Vitamin E is one of the famous antioxidants that prevent the generation of free radicals and consequently reduce the

oxidation of the meta - Pancreatic pancreas prevents the aggravation of DN [27-28].

CONCLUSION

The low level of vitamins C and E increase free radical production which is due elevated oxidative stress that leads to increased high level of MDA and thus to serious complications in diabetic patients leading to diabetic nephropathy.

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